Embryo-maternal relationships during the peri-implantation period – new and old players

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This review attempts to integrate available data on embryo-maternal communication during maternal recognition of pregnancy and implantation in the pig. Progesterone (P_4) is an essential hormone that makes the uterus receptive to accept conceptuses for implantation and subsequent placentation. As well as P_4, the receptive stage of the endometrium is further primed by paracrine factors secreted by the conceptus prior to and during implantation. Oestrogen secretion by elongating conceptuses on days 11 to 12 is necessary for the maternal recognition of pregnancy to protect the corpus luteum from regression. Oestrogens increase the expression of various uterine secretory proteins that are components of the histotroph, which in turn support conceptus growth and implantation. The PGE_2 positive feedback loop in the endometrium contributes to an increased $PGE_2/PGF_{2\alpha}$ ratio during the peri-implantation period. Greater synthesis of prostacyclin by the uterus, along with activation of the VEGF-receptor system, may be involved in regulating the uterine vascular bed and blood flow, both being prerequisite for sufficient embryo nutrition. HOXA10 expressed by the endometrium may be involved in tissue remodelling and proliferation during conceptus implantation. LIF, IL6 and their receptors are present at the maternal-conceptus interface, and both cytokines increase the attachment and proliferation of trophoblast cells. TGFβ1 stimulates the synthesis of fibronectin, which is necessary for firm adhesion between trophoblast and maternal tissue. Moreover, cytokines regulate a maternal immune response that is permissive for successful pregnancy establishment.

Introduction

The endometrium undergoes morphological and physiological changes during each oestrous cycle in order to prepare this tissue for embryo implantation. The action of progesterone (P_4) in priming the uterus is essential to attain uterine receptivity for implantation in many species, including the pig (Spencer *et al.* 2004, Ziecik *et al.* 2011). Under the influence of P_4, the endometrium is transformed into a secretory tissue to create an environment permissive to conceptus development, implantation and placentation (Spencer *et al.* 2004). P_4 stimulates the expression of vascular endothelial growth factor (VEGF; Welter *et al.* 2003), swine leukocyte antigens (SLA; MHC class I molecules in pigs) and β_2-microglobulin (β_2m; Joyce *et al.* 2008), as well as homeobox (HOX) A10, prostaglandin endoperoxide synthase 2 (PTGS2; Blitek *et al.* 2010*b*) and prostacyclin synthase (PGIS; Morawska *et al.* 2012) levels in the porcine

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endometrium. P_4 alone is able to increase vascular development in the uterus (Bailey *et al.* 2010). Interestingly, sustained P_4 action on endometrial tissue results in down-regulation of progesterone receptors (PGR) in luminal and glandular epithelium (LE and GE) beginning from day 10 after ovulation (Geisert *et al.* 1994, Ross *et al.* 2010). Mechanisms regulating PGR expression in the uterine epithelium are P_4-dependent and occur before day 6 of pregnancy (Mathew *et al.* 2011). Loss of PGR is negatively correlated with nuclear factor κβ (NFκβ) expression, which is a critical component of implantation window opening in the pig (Ross *et al.* 2010). However, the activation of NFκβ is involved in elevation of endometrial PTGS2 expression on day 12 after oestrus (Geisert *et al.* 2012), rather than in PGR down-regulation (Ross *et al.* 2010, Mathew *et al.* 2011).

In pigs, P_4 action is a prerequisite for the attainment of uterine receptivity, but developing conceptuses further modulate the endometrial milieu, making it competent for implantation. Pregnancy recognition is the result of oestrogen synthesis and secretion by conceptuses on days 11 to 12 after fertilization (Geisert *et al.* 1990). Oestrogen actions in the porcine uterus are both antiluteolytic and luteotropic. Oestradiol-17β (E_2) decreases the amount of luteolytic $PGF_{2\alpha}$ reaching the CL (Bazer & Thatcher 1977, Krzymowski & Stefanczyk-Krzymowska 2004). Moreover, E_2 stimulates luteal P_4 secretion directly (Conley & Ford 1989), and also increases the concentration of luteinizing hormone receptors in the CL (Garverick *et al.* 1982). Effects of conceptus E_2 in the uterus include regulation of PG synthesis to favour luteoprotective PGE_2 (Waclawik *et al.* 2009), stimulation of nitric oxide production (Andronowska & Chrusciel 2008) and increased uterine blood flow (Ford *et al.* 1982). Second, a more sustained peak of oestrogen release occurs between days 15 and 25-30 of gestation and is required to complete pregnancy establishment in the pig (Geisert *et al.* 1990).

Components of conceptus-maternal communication during early implantation

Implantation involves a complex series of events that establish the connection between uterine and conceptus tissues. The absence of functional PGR in LE and GE coincides with decreased expression of mucin-1 (Muc-1) on the apical surface of the uterine epithelium. Muc-1 is considered as a barrier to implantation, because reduction of its expression due to cell surface protease activity results in increased accessibility of integrin receptors to their ligands (for a review see: Bazer *et al.* 2009).

Reciprocal embryo-maternal interactions modify the local environment, enabling conceptus development and implantation. These interactions may occur either by cell-to-cell contact or through soluble mediators. Among them PGs, cytokines, growth factors and their receptors are involved (Fig. 1). Besides P_4, E_2, and PGE_2, which role in pregnancy establishment is well described, some new factors e.g. prostacyclin and HOXA10 seem to be important for embryo-maternal dialogue in pigs.

Prostaglandin E_2 (PGE_2)

The proper ratio between luteoprotective PGE_2 and luteolytic $PGF_{2\alpha}$ synthesized by the endometrium and the conceptus is essential for successful establishment of pregnancy in the pig (Waclawik 2011). Differential expression profiles for PTGS2, mPGES-1 (the microsomal isoform of PGE synthase) and PGFS (PGF synthase) were demonstrated in the endometrium of cyclic and early pregnant gilts (Blitek *et al.* 2006, Waclawik *et al.* 2006). The content of PGE_2 and $PGF_{2\alpha}$ in the uterine lumen increases progressively during early pregnancy (Table 1; Ashworth *et al.* 2006). Moreover, greater concentrations of both PGs were found in gravid

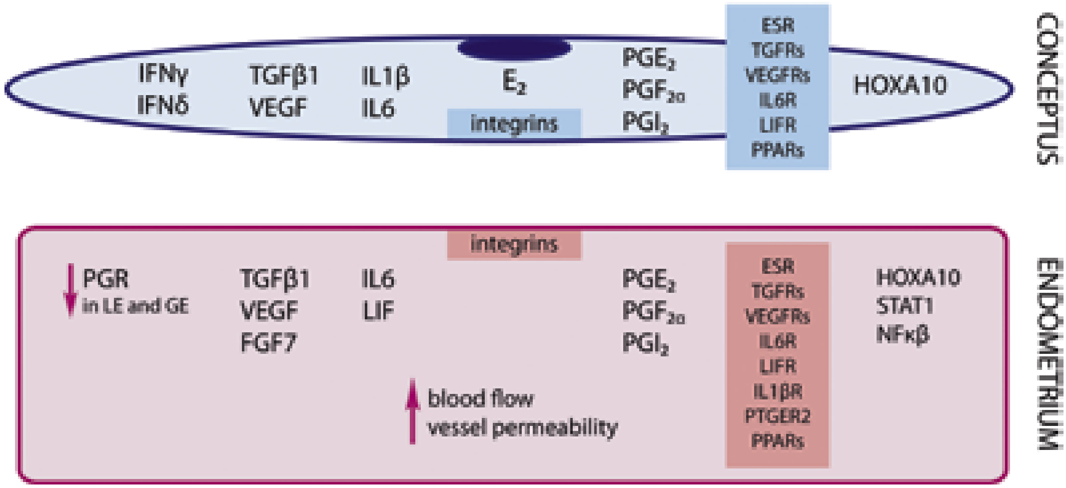

Fig. 1 Endometrial receptivity for conceptus implantation in pigs is established by the action of ovarian progesterone; nonetheless, conceptus oestradiol-17β (E_2,) further modulates the production of PGs, cytokines, growth factors, transcription regulators and integrins. All these factors acting through autocrine, paracrine and juxtacrine signaling pathways modify the local intrauterine environment, enabling conceptus development and implantation. Abbreviations: IFNγ, interferon γ; IFNδ, interferon δ; PGR, progesterone receptor; TGFβ1, transforming growth factor β1; VEGF, vascular endothelial growth factor; FGF7, fibroblast growth factor 7; IL1β, interleukin 1β, IL6, interleukin 6; LIF, leukemia inhibitory factor; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostacyclin; ESR, estrogen receptor; TGFRs, TGFβ receptors; VEGFRs, VEGF receptors; IL6R, IL6 receptor; LIFR, LIF receptor; IL1βR, IL1β receptor; PTGER2, PGE_2 receptor; PPARs, peroxisome proliferator activated receptors; HOXA10, homeobox A10; STAT1, signal transducer and activator of transcription 1; NFκβ, nuclear factor κβ; LE, luminal epithelium; GE, glandular epithelium.

than in non-gravid uterine horns on day 14 of pregnancy (Wasielak *et al.* 2009). Interestingly, E_2 up-regulates endometrial PGE_2 synthesis and the $PGE_2/PGF_{2\alpha}$ ratio by increasing PTGS2 and mPGES-1 expression, and decreasing PGFS expression (Waclawik *et al.* 2009). PGE_2 is considered to be involved in protecting the CL against the luteolytic effect of $PGF_{2\alpha}$ during early pregnancy. Additionally, elongating conceptuses are an important source of PGE_2 (Waclawik & Ziecik 2007), and PGE_2 acting through the endometrial PGE_2 receptor (PTGER2) activates a cAMP signaling pathway and elevates its own synthesis (Waclawik *et al.* 2009). Moreover, greater endometrial expression of PTGER2 during conceptus attachment (Waclawik *et al.* 2009) indicates an important local effect of PGE_2 in establishing the proper environment for embryo implantation.

Prostacyclin (prostaglandin I_2; PGI_2)

Besides PGE_2, another *PTGS*-derived prostaglandin, PGI_2, seems to be involved in pregnancy establishment. Similar to PGE_2, PGI_2 is an important regulator of vascular function. Moreover, PGI_2 is critical for blastocyst implantation in mice (Lim *et al.* 1999). Recently, we demonstrated different profiles of PGIS expression and 6-keto $PGF_{1\alpha}$ (a PGI_2 metabolite) concentration in the endometrium of cyclic and pregnant gilts (Morawska *et al.* 2012). A higher PGIS protein level and PGI_2 concentration in the endometrium observed on day 12 in pregnant gilts, followed by a greater concentration of PGI_2 in the endometrium and uterine lumen, hints at a conceptus-dependent regulation of PGI_2 synthesis. Consistent with this idea, incubation of endometrial explants with conceptus-conditioned medium stimulated PGIS protein expression

Table 1. Profiles of E_2, calcium, total protein, prostaglandins and cytokines concentration in the uterine lumen of the pig during the peri-implantation period.

	Days of pregnancy					*References*
	9-10	*11-12*	*13-14*	*15-16*	*17-18*	
E_2	●	●●●●●	●	●●●	NR	Stone & Seamark 1985
Calcium	●	●●●●●	●	●	●	Geisert *et al.* 1990
Total protein	●	●●	●●●	●●●●	●●●●●	Geisert *et al.* 1990
IFNs	○	●	●●●●	●●●●	●●●●	La Bonnardière *et al.* 1991
IL1β	●	●●●●	●●●●	●●●●	●●	Ashworth *et al.* 2006
IL6	●●	●●●●	●●	NR	NR	Anegon *et al.* 1994
LIF	●	●●●●	●●	●	NR	Anegon *et al.* 1994, Blitek *et al.* 2012
TGFβ1	○	●●●	●●●	NR	NR	Gupta *et al.* 1998
PGE_2	●	●●	●●●	●●●●	●●●●●	Ashworth *et al.* 2006
$PGF_{2\alpha}$	●●	●	●	●●●	●●●	Ashworth *et al.* 2006
PGI_2	●	●●	NR	●●●●	●●●●	Morawska *et al.* 2012

● – level of concentration; ○ – not detected; NR – not reported
Numbers of black dots are used to represent how the concentration of selected molecule changes during the peri-implantation period; they do not reflect the exact fold-change between days or particular molecules.

and PGI_2 release. Furthermore, using an *in vivo* model of gilts with unilateral pregnancy, we demonstrated that conceptus presence up-regulated PGI_2 synthesis in the endometrium on day 14 of gestation (Morawska *et al.* 2012). Greater synthesis of PGI_2 on day 18 in pregnant gilts may be associated with regulation of blood flow, which is essential for sufficient foetal nutrition. PGI_2 is also synthesized by pig conceptuses (Morawska *et al.* 2012). PGI_2 not only elicits its effect on target cells via a cell surface PGI receptor, but may also act via nuclear peroxisome proliferator activated receptors (PPARs). The expression of PPARs has been demonstrated in porcine endometrium and trophoblast tissue (Lord *et al.* 2006). Thus, an effect of PGI_2 on the permeability of maternal and conceptus vasculature is very likely. Moreover, treating porcine blastocyst with PGI_2 resulted in higher numbers of inner cell mass and trophectoderm cells compared with nontreated blastocysts (Kim *et al.* 2010).

Homeobox A10 (HOXA10)

Transcription factors in the endometrium are regulated by ovarian P_4 and/or embryonic stimuli and thus may be critical for implantation. One such factor that plays an important role in uterine physiology is HOXA10. In the porcine uterus, HOXA10 protein was localized in LE, GE, stromal cells and blood vessels. Endometrial expression of HOXA10 increases progressively during early pregnancy, and day 15 pregnant gilts express a 2-fold higher level of *HOXA10* transcripts than their cyclic counterparts (Blitek *et al.* 2010*a*, 2011). These results indicate that conceptus presence may induce *HOXA10* gene expression in the porcine endometrium, but also confirm a role of HOXA10 during the implantation period, rather than at the time of maternal recognition of pregnancy. It seems likely that HOXA10 is involved in uterine remodelling to support placentation. Ovarian steroids, P_4 and E_2, as well as conceptus-derived products, stimulate the expression of *HOXA10* in porcine endometrial cells (Blitek *et al.* 2010*b*, 2011), thereby probably promoting proliferation and differentiation of endometrial cells during implantation. A role of HOXA10 in a local immunomodulation within the uterus has also been postulated (Yao *et al.* 2003). Interestingly, increased *HOXA10* mRNA levels in response to E_2 and conceptus products were accompanied by greater *PTGS2* gene expression and PGE_2 secretion from endometrial cells (Blitek *et al.* 2011).

Leukemia inhibitory factor (LIF) and interleukin 6 (IL6)

LIF and IL6 are present within the uterine microenvironment during early pregnancy. Endometrial expression of *LIF* mRNA changes during peri-implantation period, with the lowest level observed on day 10 followed by a sharp increase on day 12. Moreover, *in vitro* experiments revealed that the addition of conceptus-exposed medium to endometrial explants results in greater *LIF* mRNA content in the tissue (Blitek *et al.* 2012). Nevertheless, endometrial *LIF* expression increases also in cyclic gilts, suggesting that besides a conceptus effect, maternal regulation of LIF synthesis occurs in the pig uterus. A dramatic increase in LIF protein content in the uterine lumen is observed between days 10 and 12 of pregnancy (Table 1; Anegon *et al.* 1994, Blitek *et al.* 2012). The main source of high LIF concentrations in the uterine lumen is the endometrial tissue, because no (Modrić *et al.* 2000) or very low (Blitek *et al.* 2010*a*) *LIF* mRNA expression was detected in conceptuses. Greater expression of IL6 protein in the endometrium (Blitek *et al.* 2012) as well as IL6 concentration in the uterine lumen (Anegon *et al.* 1994) was observed in pregnant than in cyclic gilts. Peak expression of *IL6* mRNA in the endometrium was detected on day 12 of pregnancy and was accompanied by higher IL6 protein content. Similar to LIF, IL6 synthesis in the porcine endometrium seems to be stimulated by products of conceptus origin (Blitek *et al.* 2012). In contrast to LIF, pig conceptuses are an important source of IL6 (Modrić *et al.* 2000). Moreover, specific cytokine receptors, LIFR and IL6R, have been detected in the endometrium (Modrić *et al.* 2000) and conceptuses (Blitek *et al.* 2012). The attachment and proliferation of trophoblast cells are both stimulated by LIF and IL6 (Blitek *et al.* 2012). Additionally, IL6 activates endometrial $PGF_{2\alpha}$ release and metabolism in a manner that is consistent with protection of the CL against regression (Franczak *et al.* 2012).

Vascular endothelial growth factor (VEGF) and the vascular system

Development of the placental vascular architecture is of considerable importance in the exchange of nutrients, oxygen, and carbon dioxide between the mother and fetus. In pigs, increased uterine arterial blood flow was observed on days 12-13 of gestation close to the embryonic disc. It was accompanied by elevated vascular permeability, leading to edema of the endometrium (Krzymowski & Stefanczyk-Krzymowska 2004, Ziecik *et al.* 2011). Most of the vascular changes are initiated after the first embryonic signal, however, VEGF is thought to be an effective component of this system. Interestingly, increased expression of VEGF164 protein was observed on days 10-15 of pregnancy, coincident with the time of maternal recognition of pregnancy and major vascular events of the peri-implantation period in pigs (Kaczmarek *et al.* 2008*b*). Both insulin-like growth factor I and relaxin seem to be potent inducers of VEGF in the porcine uterus. Endometrial synthesis of VEGF164 by day 15 of pregnancy can be also stimulated by PGE_2 (Kaczmarek *et al.* 2008*a*). Identification of VEGF164 expression in conceptuses on days 12 and 15-16 (Kaczmarek *et al.* 2009) suggests that attachment-associated changes in the vascular compartment and in endometrial morphology can be directed by this factor acting in a local manner.

Other factors involved in conceptus-endometrial interactions

Interleukin 1β (IL1β) and its signalling system are expressed in the endometrium and conceptuses of the pig, and IL1β may be important for immunotolerance at the maternal-placental interface.

Conceptus synthesis and secretion of IL1β occurs within a short time window associated with trophoblast elongation between days 11 and 12 of pregnancy (Ross *et al.* 2003). It is supposed that IL1β acting in an autocrine manner may induce the morphological transformation of trophectoderm during the process of elongation (Geisert *et al.* 2012). In the endometrium, IL1β stimulates PTGS2 and mPGES-1 expression and PGE_2 content (Franczak *et al.* 2010). Thus, conceptus IL1β may participate in blocking luteolysis by increasing PGE_2 production, as well as in establishment of a pro-inflammatory environment in the uterus.

Pig conceptuses produce interferon γ (IFNγ) and IFNδ during the peri-implantation period (Joyce *et al.* 2007), and IFNs activity in the uterine lumen increases significantly between days 12 and 16 of pregnancy (La Bonnardière *et al.* 1991). Neither of the IFNs produced by pig conceptuses has antiluteolytic activity, but their paracrine action in the endometrium has been demonstrated (Joyce *et al.* 2007, 2008). Interestingly, E_2 stimulates expression of the signal transducer and activator of transcription (STAT) 1 in LE cells, while stromal expression of STAT1 is up-regulated by IFNs (Joyce *et al.* 2007). Moreover, down-regulation of SLA molecules and β_2m observed in endometrial LE during implantation may be crucial for preventing foetal allograft rejection. In contrast to LE, expression of SLAs and β_2m in the stroma increases in response to conceptus IFNs (Joyce *et al.* 2008). Thus, IFNs seem to be important components of the immune system involved in preparation of an environment suitable for conceptus implantation.

Transforming growth factor β (TGFβ) participates in angiogenesis, apoptosis, proliferation, immunotolerance, embryogenesis and tissue remodelling (Godkin & Dore 1998), and all these processes are important during the peri-implantation period. TGFβ isoforms and its type I and II receptors were localized at the maternal–conceptus interface in the pig. Moreover, the amount of biologically active TGFβs in the uterine lumen increases significantly during the peri-implantation period (Gupta *et al.* 1998). Localization of phosphorylated SMAD2/3 proteins in conceptus and uterine tissues indicates activation of TGFβ-dependent intracellular signalling, possibly leading to conceptus development and attachment (Massuto *et al.* 2010). Importantly, TGFβ1 enhances the expression of fibronectin and activates integrin receptors (Jaeger *et al.* 2005), but also stimulates proliferation of porcine trophoblast cells (A. Blitek, unpublished).

Conclusion

Loss of PGR in the uterine epithelium during the peri-implantation period is associated with changes in endometrial gene expression leading to achievement of uterine receptivity for conceptus implantation. In response to conceptus signals, the uterus secretes large amounts of a wide variety of biological factors, and these secretions establish a unique embryotrophic environment that plays an important role in the survival of embryos as well as in implantation and placentation. However, detailed mechanisms involved in reciprocal embryo-maternal relationships are yet to be elucidated. Identification of specific downstream genes/proteins/signalling pathways regulated by components of the histotroph might be helpful in better understanding of embryo-maternal interactions, not only in the pig but also in other species with diffuse type of placenta. Identification of genetic markers of embryo signalling and endometrial receptivity during the peri-implantation period would be of great benefit for increasing reproductive efficiency, and also for optimizing transgenesis, cloning and other biotechniques.

Declaration of interest

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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